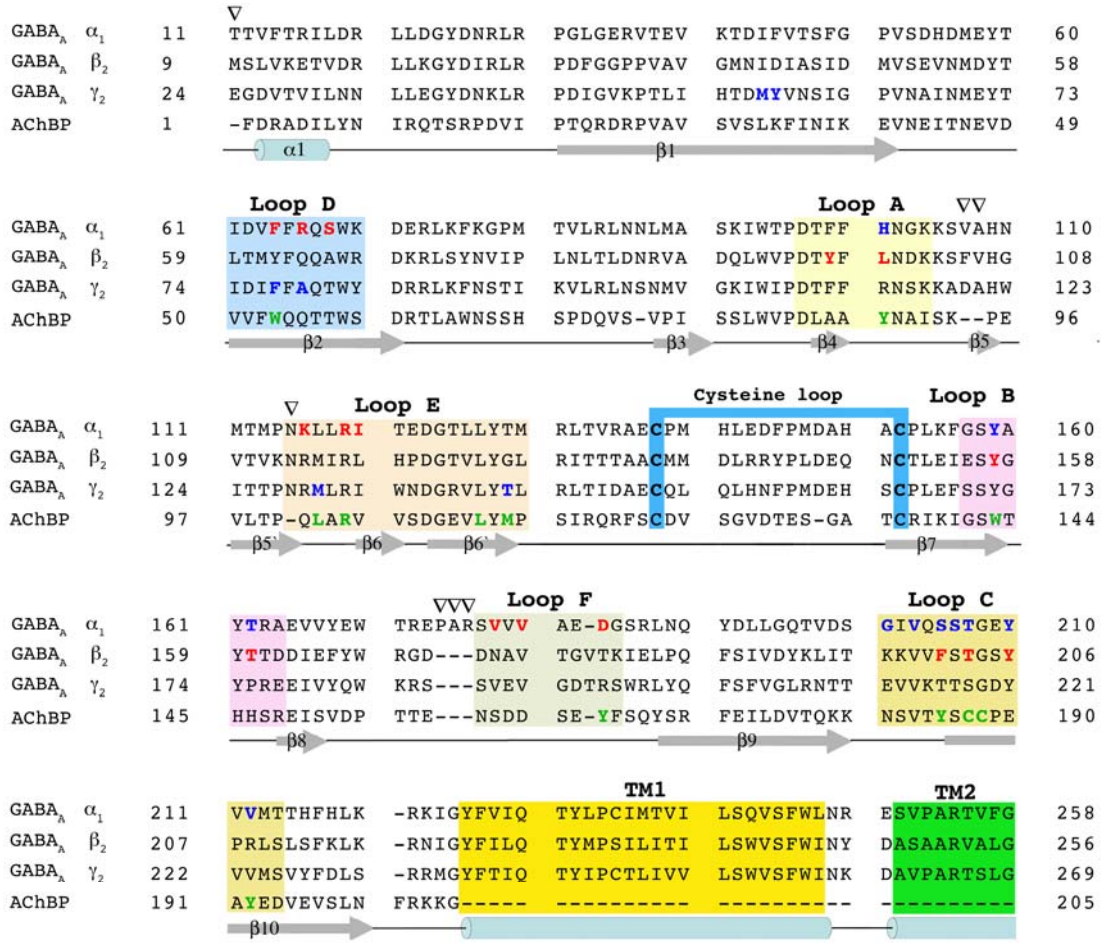
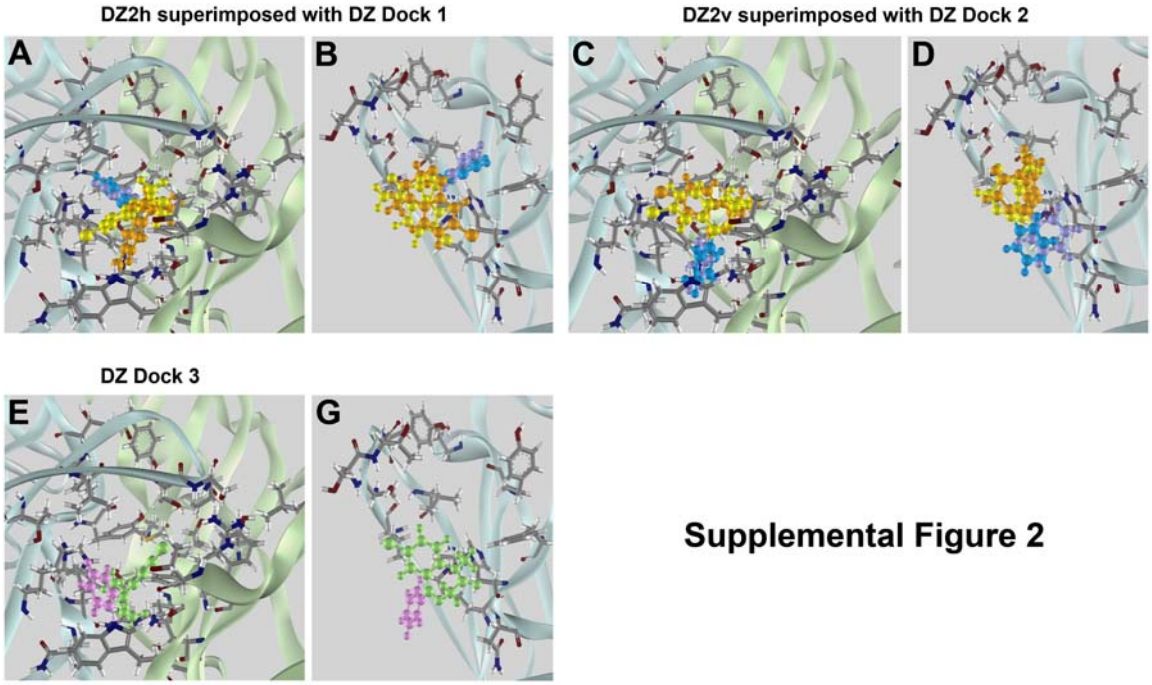
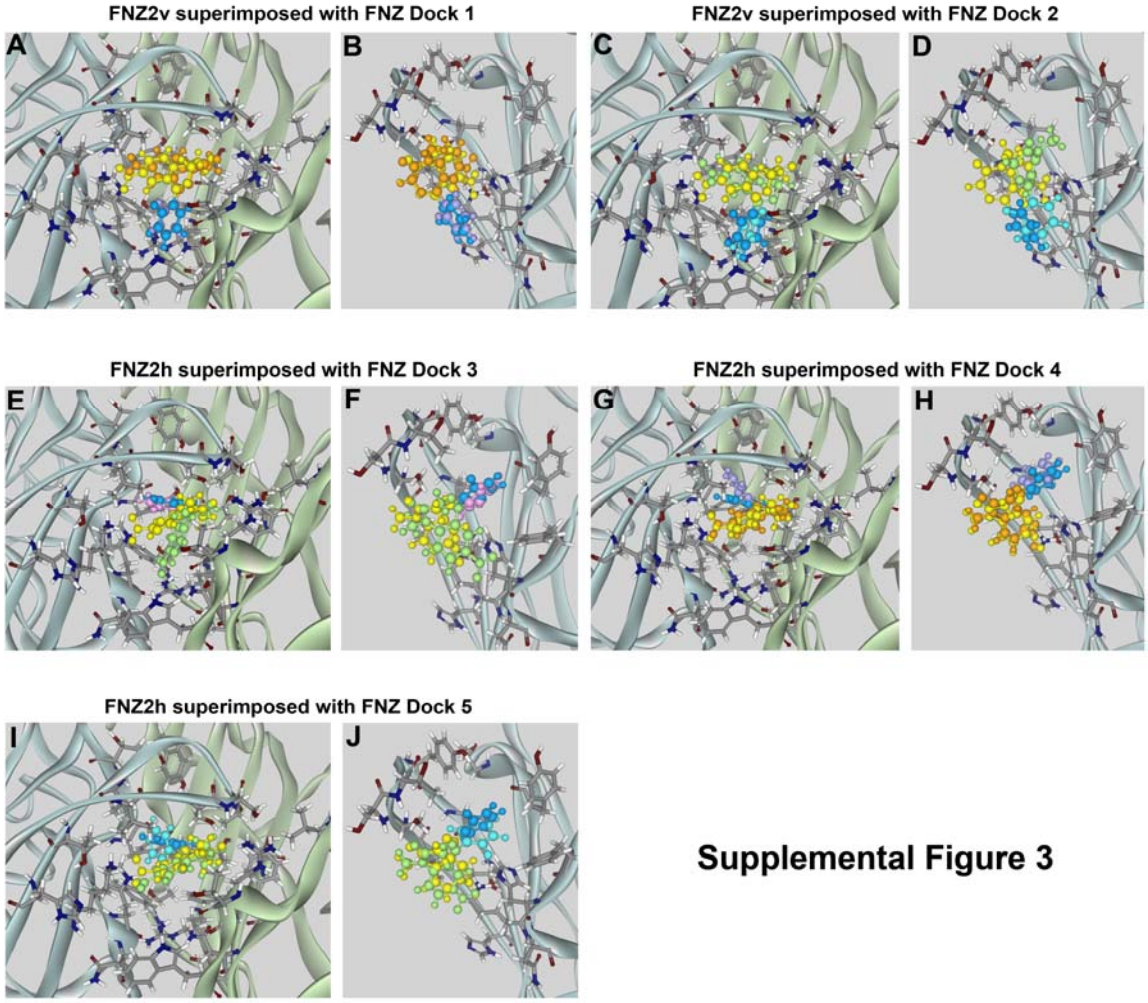


Supplemental Figure 1

Supplemental Figure 1. Alignment of the ligand-binding domain of the $GABA_AR$ α_1, β_2 and γ_2 subunits and AChBP. Residues forming the cysteine loop are highlighted in blue. The secondary structure is represented by barrels (helices) and arrows (β-sheets) pictured underneath the alignment. Colored highlighting indicates six regions thought to play a role in ligand binding: loops A, B, C, D, E and F. Residues that are thought to contribute to the agonist recognition site are in red and those thought to contribute to the BZD binding site are in blue. Residues within a radius of 5 Å of nicotine molecules in the AChBP structure are indicated in green (Brjec et a., 2001). The ∇ symbol indicates structurally ambiguous areas of alignment of the $GABA_AR$ subunits and AChBP. Precursor peptide sequences of rat subunits were obtained from NCBI (acc. codes: P18504 for alpha1; P15432 for beta2; P22723 for gamma2). Ligand binding domains were aligned using the Align123 algorithm from Discovery Studio software package.



Supplemental Figure 2. Superposition of orientations of DZ obtained by manual and automated docking (**A-G**). For DZ there is a good overlap between DZ2h (orange-blue) and DZ dock 1 (yellow-violet) (**A**, **B;** RMSD = 1.8Å) and between DZ2v (orange-blue) and DZ dock 2 (yellow-violet) (**C**, **D**; RMSD = 1.36Å). The DZ dock 3 (green-pink) is also depicted. Pairs of images depict the BZD binding pocket viewed from outside of the receptor (**A**, **C, E**) and from within the binding pocket looking toward the α subunit (**B**, **D, G**).



Supplemental Figure 3

Supplemental Figure 3. Superposition of FNZ orientations obtained via manual and automated docking (**A-J**). Automated docking orientations FNZ dock 1 (**A** and **B**, orange-violet) and FNZ dock 2 (**C** and **D**, green-cyan) resemble manual docking orientation FNZ2v (yellow-blue) with respective RMSD of 0.81Å and 3.04Å. Similarly, automated docking orientations FNZ dock 3 (**E** and **F**, green, pink), FNZ dock 4 (**E** and **F**, orange-violet) and FNZ dock 5 (**I** and **J**, green-cyan) resemble manual orientation FNZ2h (**G** and **H**, yellow-blue) with respective RMSD of 3.82 Å, 1.06Å and 1.46Å. Pairs of images depict the BZD binding pocket viewed from outside of the receptor (**A**, **C**, **E**, **G, I**) and from within the binding pocket looking toward the α subunit (**B**, **D**, **F**, **G, J**).

	Ligands	Energy, (kcal/mol)	Population, %
MANUAL DOCK	DZ1h	-110	100%
	DZ1v	-96	100%
	DZ2h#	-78	100%
	DZ2v##	-77	100%
	FNZ1h	-329	100%
	FNZ1v	-314	100%
	FNZ2h*	-275	100%
	FNZ2v**	-286	100%
CDOCKER DOCK	DZ dock1#	-58	35%
	DZ dock2##	-68	35%
	DZ dock3	-38	30%
	FNZ dock1**	-249	30%
	FNZ dock2	-228	20%
	FNZ dock3	-160	25%
	FNZ dock4*	-255	5%
	FNZ dock5*	-266	20%

Supplemental Table 1. DZ and Flu docking energies (kcal/mol). Manual docking of DZ (DZ1h, DZ1v, DZ2h, DZ2v) and FNZ (FNZ1h, FNZ1v, FNZ2h, FNZ2v) docking simulations was carried out as detailed under *Materials and Methods*. Automated docking using CDocker yielded three orientations of DZ (DZ dock1-3) and five orientations of FNZ (FNZ dock1–5). Potential energies were calculated using Calculate Interaction Energy protocol. Symbols (#, ##, *, **) identify similar models.

Residue (atom number)	Distance, Å	Chain (Flu-2h)/Atom ID
α1F99 (HE1)	3.8	F-H (7441)
α1H101 (HD1)	2.1	F-N (7433)
α1N102 (HA)	6.9	F-O (7448)
α1K155 (HZ3)	1.7	F-O (7447)
α1Y159 (HA)	2.3	F-H (7441)
α1T162 (HA)	7.7	F-H (7441)
α1G200 (O)	7.2	F-O (7447)
α1V202 (HG11)	3.1	F-O (7447)
α1S204 (HG)	1.8	F-O (7431)
α1S205 (HN)	2.8	F-O (7431)
α1T206 (HG23)	3.3	F-N (7423)
α1Y209 (HH)	5.0	F-H (7442)
α1V211 (HG11)	2.6	F-H (7440)
α1T213 (H21)	3.8	F-O (7431)
γ2Y58 (HD1)	3.0	F-H (7438)
γ2N60 (OD1)	5.3	F-H (7437)
γ2D75 (OD1)	9.3	F-H (7437)
γ2F77 (HE2)	4.1	F-H (7437)
γ2A79 (HB2)	6.9	F-F (7443)
γ2T126 (HG1)	5.9	F-H (7441)
γ2M130 (HE1)	5.4	F-H (7442)
γ2T142 (HG21)	4.5	F-F (7443)
γ2R144 (HH12)	6.9	F-O (7448)
γ2V188 (HG11)	5.7	F-O (7431)
γ2T193 (HG22)	2.3	F-H (7444)
γ2R194 (HH11)	1.6	F-O (7431)
γ2W196 (HE1)	5.6	F-H (7434)

Supplemental Table 2. Interatomic distances between closest pair of atoms belonging to the binding site residue and that of Flu were measured by 'Monitor Distance' tool of Discovery Studio using the pdb file with 2h orientations.